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How to track genetically modified (GM) plants in the field? The VDI standard method of floristic mapping of GM plants as an efficient tool

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Abstract

The commercial use of genetically modified (GM) organisms is regulated in the EU by law. Thus, monitoring the environmental effects of GM organisms after placement on the market is a mandatory task of the respective consent holder. Since many relevant monitoring procedures lack standardisation, the Association of German Engineers (VDI) has commissioned expert groups with the development of guidelines covering appropriate methodologies. As part of this project, the VDI Guideline 4330 Part 10 was set up (Bleeker et al. 2011) describing a standardised procedure for floristic mapping of spontaneously occurring (non-cultivated) GM crops, their wild potential crossing partners and their hybrid offspring. Areas to be mapped are those where such plants are expected to be found, e.g. on former fields and in the vicinity of current or former fields of GM plants. In the case of transportation, processing or use of GM plants as animal feed, these are areas surrounding the processing, storage, handling and usage facilities, including access routes to and from the facilities. The concept of adverse environmental effects caused by the dispersal and outcrossing of GM plants is briefly introduced. The necessity of floristic mapping in the context of post-market environmental monitoring of GM plants is demonstrated taking oilseed rape as an example. The development of the Guideline VDI 4330 Part 10 is described and its contents are summarised. An important conclusion on the relevance and efficiency of the floristic mapping method is that strict standardisation ensures a high level of EU wide reproducibility and comparability of the results.

Keywords

Genetically modified plants, post-market environmental monitoring, VDI guidelines, standardised methods, floristic mapping, wild crossing partners, hybrid offspring, oilseed rape, *Brassica napus*

Introduction

The commercial use of GM organisms may have various environmental impacts at the level of species, habitats or ecosystems (Sukopp and Sukopp 1993, Letourneau and Burrows 2002, Velkov et al. 2005, Dolezel et al. 2007, Bartz et al. 2009, EFSA 2010). This happens for example, if GM crops spontaneously spread and persist outside cultivation or cross with other sexually compatible plants (Ellstrand and Hoffman 1990, Nijs et al. 2004, Pilson and Prendeville 2004, Gressel 2005, FitzJohn et al. 2007, Wilkinson and Ford 2007). Therefore, the commercial use of GM organisms is regulated in the EU by law in order to prevent adverse environmental effects caused by such use. According to Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (EC 2001) and to Regulation (EC) No 1829/2003 on genetically modified food and feed (EC 2003), monitoring the environmental effects of GM organisms after placement on the market is a mandatory task of the respective consent holder. Before placing on the market any GM organism has to pass an approval procedure led at present by the Directorate-General for Health and Consumers of the European Commission. In the application, monitoring procedures have to be described in a monitoring plan in detail. Once the application is consented, the monitoring plan has to be fully implemented with reporting obligations at regular intervals.

According to Directive 2001/18/EC, post-market environmental monitoring has to follow standard methodologies, wherever available and appropriate (see also EC 2002, EFSA 2011, BfN et al. 2011). Since many relevant monitoring procedures are not standardised so far, there is a strong need to newly develop suitable standards (Berhorn et al. 2005). For this reason, the Association of German Engineers (VDI) as an independent technical standardisation body has commissioned several expert groups with the development of so called VDI Guidelines covering appropriate monitoring methodologies (see Züghart et al. 2013). The scientific and administrative experts discussed and decided on needs and priorities of standardised methods. The outcome are specific regulations partly published and partly still in progress (see overview on Guidelines VDI 4330 – 4333 in Tab. 2 and Tab. 3 in Züghart et al. 2013). The exact description of monitoring techniques should enable the staff to collect and analyse data in a scientifically and statistically sound manner as required for the post-market environmental monitoring of GM organisms (EC 2002).

Technical guidelines facilitate monitoring activities since they provide standardised methods ensuring data quality and comparability over time and space (Graef et al. 2007). This is particularly important if data are collected in large areas and over longer periods of time. Post-market environmental monitoring of GM organisms is expected to last at least 10 years and may cover the entire EU territory. Therefore, comparison of data relies on highly standardised field methods. This applies also for floristic mapping data if required.

In our contribution, we explain the necessity of floristic mapping in the context of post-market environmental monitoring of GM plants that may spread and persist or form hybrid offspring. Further, we address the question of adverse environmental

effects caused by the dispersal and outcrossing of GM plants. Oilseed rape serves as a prominent example of a common GM crop that can be monitored using standardised procedures. Development and contents of the Guideline VDI 4330 Part 10 on floristic mapping (Bleeker et al. 2011) are briefly explicated. Finally, conclusions are drawn on the relevance and efficiency of the VDI standard method of floristic mapping.

Floristic mapping in the context of post-market environmental monitoring of GM plants

Floristic mapping is the spatial and temporal recording and subsequent cartographic representation of the occurrence of plants in the landscape. For this purpose, the name of the plant taxon and the locality must be determined. Furthermore, as a minimum requirement, the observation date and the name of the observer must be recorded. The resulting data can be stored in a data bank or can be shown on a map of the study area.

The use of GM plants can comprise cultivation including transport of seed and harvest material to and from fields. If GM plants are used for the production of food or feed, GM raw material as well as processed commodities containing GM material are transported between storage yards, harbours, railway stations, reloading areas and processing plants. During cultivation, transport, storage, handling or processing of GM plants, viable plant material (e.g. seeds, fruits, tubers) can get into the environment e.g. through incidental spillage. If this is leading to spontaneously growing and reproducing GM plants, further events can be triggered: (1) ongoing spontaneous dispersal of GM plants, (2) establishment of persistent populations of GM plants, (3) outcrossing of GM plants into potential crossing partners occurring in the wild, (4) establishment and spontaneous dispersal of hybrid offspring resulting from those crosses. Outcrossing and the production of hybrid offspring can occur also via pollen transfer from GM crops to wild crossing partners.

Since the early 1990s, concepts have been elaborated including monitoring methods for the above mentioned environmental effects of GM plants in various European countries (see among others Sukopp and Sukopp 1993, 1997, Traxler et al. 2001, Züghart and Breckling 2003, Heissenberger et al. 2003, ACRE 2004, Züghart et al. 2005, 2008, Pascher et al. 2010, EFSA 2011, BfN et al. 2011). In Germany, the first projects developing and testing suitable floristic field methods started at the end of the 20th century. Theenhaus et al. (2005) developed monitoring methods for a Bavarian landscape between Neustadt an der Donau and Kelheim including floristic mapping of Brassicaceae species as potential crossing partners of oilseed rape and sampling of leaf material for molecular biological analysis. Menzel (2006) investigated the spread of conventional oilseed rape and the distribution of potential crossing partners in and around the city of Bremen. Similar surveys were carried out by Haeupler et al. (2004) in the region around Detmold (North Rhine-Westphalia) and by Elling (2008) in the region around Osnabrück (Lower Saxony).

The State Agency for Nature, Environment and Consumer Protection of North Rhine-Westphalia is so far the only German state authority having implemented a

regular monitoring programme regarding the potential spread of GM plants in the environment (König 2009). In 2004, the observations started as part of a much broader biodiversity monitoring programme. Sampling of leaf material of spontaneously occurring oilseed rape and eight potential crossing partners is carried out in representative parts of the landscape. The molecular biological analysis proved the presence of DNA sequences of GM oilseed rape in three samples in 2004 (König 2009).

GM plant dispersal and formation of hybrid offspring

The success of spontaneous GM plant dispersal and outcrossing events highly depends on many different influencing factors and conditions. Among these factors are: (1) the level of cross-pollination, (2) the degree of allogamy, (3) the level of domestication of the GM crop, (4) the presence and frequency of sexually compatible wild plants, (5) the amount of production of hybrid seeds, (6) the selective advantage of certain new traits of the hybrid plants, (7) the availability of suitable sites for the establishment of hybrid plant populations (Ellstrand and Hoffman 1990, Sukopp and Sukopp 1994, Bleeker et al. 2007). The resulting dispersal of GM plants or of their hybrid offspring can be recorded in the field with the help of floristic mapping. The probability of the before mentioned processes highly increases in the presence of crop-weed-complexes (Harlan 1965, 1982, Hammer 1991). In many cases, both crops and weedy relatives are the result of a very similar evolutionary process. Most crop species are known to have formed at least in some parts of the world closely related weedy forms that can easily produce offspring with the respective crop variety. In English literature, this phenomenon is named crop ferality (Gressel 2005). Crop-weed-complexes are described e.g. for sugar beet (Desplanque et al. 1999, Soukup and Holec 2004, Sukopp et al. 2005), oilseed rape (Hall et al. 2000, 2005, Salisbury 2002, FitzJohn et al. 2007) and bread wheat (OECD 1999, Zaharieva and Monneveux 2006).

Adverse environmental effects caused by GM plant dispersal and formation of hybrid offspring

Floristic mapping in the context of post-market monitoring of the environmental effects of GM plants and their use is triggered by (1) the hypothesis that viable GM plant material may spread or (2) by the hypothesis that GM plants may outcross with other sexually compatible plants. While the first hypothesis applies to all commercial uses of viable GM plants or viable GM plant material (e.g. seeds), the second hypothesis presupposes the presence of sexually compatible crossing partners. If there are so far no crossing partners known in the area under survey, monitoring according to the second hypothesis is not necessary. An example for a common crop plant of the last type in Central Europe are potatoes. However, if crossing partners may occur in the study area, the second hypothesis specifically triggers the search for such crossing partners and for potential hybrid offspring (Sukopp and Sukopp 1993, 1994, 1997, Sukopp and Weddeling 2007, Bleeker

et al. 2007, Schmitz et al. 2008). It should be noted, that the GM plants can be used (e.g. cultivated or processed) either directly in the area under survey or in other more or less remote regions. In the last case, the GM plants may be – most often – accidentally introduced into the study area by means of transportation as it was documented for oilseed rape in Canada (Yoshimura et al. 2006), in Japan (Aono et al. 2006, Kawata et al. 2009, Nishizawa et al. 2009), in the USA (Schafer et al. 2011) and in Switzerland (Schoenenberger and D'Andrea 2012). In Guideline VDI 4330 Part 10, lists of known crossing partners of two common European crops are presented (Bleeker et al. 2011). These refer to oilseed rape (*Brassica napus*) and to bread wheat (*Triticum aestivum*). Both lists contain ephemeral, established or cultivated species found in Germany of which at least one successful spontaneous crossing or one successful experimental crossing using hand pollination with the respective crop species is known to have occurred in or outside Germany.

Post-market environmental monitoring aims at detecting adverse effects of GM plants and their use on human health and the environment. Such adverse environmental effects or damages occur when a relevant conservation resource is significantly adversely affected (for the development of this definition see Sukopp and Sukopp 1993, 1994, Bartz et al. 2005, 2009, Kowarik et al. 2008, Heink et al. 2012). The identification of a significant adverse effect comprises the determination of its magnitude and of the value of the affected conservation resource. For the implementation of this concept, suitable criteria must be found and thresholds must be agreed on (Kowarik et al. 2008). The magnitude of adverse environmental effects is affected by the extent of persistence and spread of GM plants, by the frequency of outcrossing events and by the extent of persistence and spread of the resulting hybrid offspring. All these events and processes alone do not determine a damage to conservation resources. According to a cause-effect-hypothesis, such events can trigger other events that may finally cause a damage e.g. if escaped hybrid offspring can establish highly dominant populations which replace populations of a protected native plant species. In this example, a certain conservation goal is significantly impaired (for further details of the concept see Kowarik et al. 2008). Since outcrossing of GM plants or the spread and persistence of GM plants or hybrid offspring can be a step towards adverse environmental effects, post-market environmental monitoring of GM plants has to address those processes. The monitoring results should help confirm or reject the initial hypothesis concerning outcrossing, persistence and spread of the GM plants and of potential hybrid offspring (BfN et al. 2011). In the context of the environmental risk assessment of GM plants, it has to be considered that future fitness estimations do not only depend on genotypes but rather on the interactions between a particular plant phenotype and its specific environment. Since future environmental changes are not fully predictable, a substantial uncertainty remains in such fitness estimations. Therefore, according to the precautionary principle, the persistence of a population of GM plant individuals can already be an issue of concern (see Kowarik et al. 2008).

Taking oilseed rape as an example, Breckling et al. (2003) and Breckling and Menzel (2004, 2005) discussed the need to consider potential adverse effects on conservation resources caused by the commercial use of GM oilseed rape in risk research, risk assessment and for post-market environmental monitoring.

Oilseed rape as an example of spread, persistence and outcrossing of a common crop

Oilseed rape is a well-known example of a widespread crop species that generates large numbers of volunteers on arable land, easily spreads to suitable habitats outside crop land and hybridises with sexually compatible wild relatives. Feral (spontaneous or subspontaneous) populations of oilseed rape and its hybrids can originate from three main sources: (1) land cultivated with oilseed rape, (2) places where oilseed rape is handled, stored or processed such as oil mills and harbours, (3) from accidental spillage during transport along roads, railways, rivers or canals.

Oilseed rape (*Brassica napus*) is a close relative of a number of other crop species (e.g. *B. rapa*, *B. oleracea*) and wild species of the Brassicaceae family (e.g. *Sinapis arvensis*, *Raphanus raphanistrum*) and can spontaneously produce fertile offspring with several of those species (e.g. Scheffler and Dale 1994, Lefol et al. 1996, 1997, Salisbury 2002, Warwick et al. 2003, Chèvre et al. 2004, Knispel et al. 2008, Elling et al. 2010). A recent comprehensive overview on hybridisation within *Brassica* and allied genera is given by FitzJohn et al. (2007). After the year 2000, first evidence of outcrossing events under field conditions with GM oilseed rape as the gene donor and other crops or weedy species of arable land as receptors was published (e.g. hybridization between GM oilseed rape and *Brassica rapa* or *Sinapis arvensis*; Halfhill et al. 2004, Daniels et al. 2005).

Conventional and GM oilseed rape can establish feral (spontaneous or subspontaneous) populations on various types of non-cropped land (Pessel et al. 2001, Loos et al. 2004, Yoshimura et al. 2006, Elling 2008, Elling et al. 2009, Kawata et al. 2009, Nishizawa et al. 2009, Schafer et al. 2011, Schoenenberger and D'Andrea 2012). These populations occur frequently along transportation routes (e.g. on roadsides, railway ground, waste ground in harbours) and close to processing plants (e.g. oil mills, crushing facilities). The spatial and temporal dynamics of populations and seed dispersal along roads were described in detail for feral oilseed rape on the M25 motorway around London (Crawley and Brown 1995, 2004). A similar study on seed spillage and seed dispersal of various plant species including oilseed rape was conducted along a motorway tunnel in Berlin (von der Lippe and Kowarik 2007a, 2007b). In France and Germany, case studies have shown that feral oilseed rape probably establishes persistent populations that do not or only partly depend on seed recruitment from cultivated populations (Pessel et al. 2001, Loos et al. 2004, Pivard et al. 2008).

Pessel et al. (2001) identified relict plants of a no longer marketed oilseed rape variety which had persisted on road verges for at least 8 years according to farmer surveys in the region of Loir-et-Cher in France. In the same region, Pivard et al. (2008) conducted a large-scale study on factors explaining the occurrence of feral oilseed rape populations on field margins and road verges. Two dominant factors were identified: (1) annual seed dispersal from surrounding fields cropped with oilseed rape, (2) persistence of feral populations, mainly through persistent seed banks. Therefore, the seed bank contribution to the dynamics of feral oilseed populations needs to be considered more seriously. Persistent seed banks combined with selfrecruitment indicate a high

potential for the persistence also of feral GM oilseed rape outside fields. Loos et al. (2004) described feral populations of oilseed rape on waste land in the Ruhrgebiet in Germany that are presumably persistent (naturalisation in anthropogenic habitats outside arable land). Elling (2008) showed that 79,5 % of the 78 examined feral populations of *Brassica napus* in the region of Osnabrück, Germany, persisted for more than one year on the same locality. In Austria, Pascher et al. (2010) demonstrated significant genetic differences between commercial varieties and feral populations of oilseed rape. The authors concluded that feral oilseed rape is able to maintain persistent populations that are already to a certain extent genetically separated from the crop varieties. At present, evidence from several European studies strongly supports the assumption that both conventional and GM feral oilseed rape persists in many places outside agricultural land (Squire et al. 2011).

Other studies have reported feral GM oilseed rape plants or populations along transport routes and in harbour facilities in Canada (Garnier & Lecomte 2006, Yoshimura et al. 2006, Knispel et al. 2008, Knispel and McLachlan 2009), Japan (Saji et al. 2005, Aono et al. 2006, Kawata et al. 2009, Nishizawa et al. 2009, 2010) and most recently also in the USA along roadsides outside the oilseed rape cultivation area (Schafer et al. 2011). Potential selective advantages of feral GM herbicide resistant oilseed rape can occur along transport routes (e.g. roadsides, railway ground) and in harbours particularly where complementary herbicides are applied. Schafer et al. (2011) described spontaneous GM oilseed rape populations at roadsides that had recently been mowed or treated with herbicides. The presence of stacked herbicide resistance genes from different cultivated GM oilseed rape varieties was observed in feral oilseed rape populations outside fields in western Canada giving evidence for gene flow among those feral populations (Knispel et al. 2008).

While observing the spread of oilseed rape along railways in Switzerland, Schoenenberger and D'Andrea (2012) proved the presence of GM herbicide resistant oilseed rape on railway ground in Basel and Lugano. The findings indicate that certain GM oilseed rape varieties may be capable of establishing persistent populations outside agricultural land, e.g. on herbicide-treated railway tracks, on handling areas and in surrounding disturbed habitats. The question arises if this leads to a transfer of the introduced herbicide resistance genes to wild species that are sexually compatible.

Development of Guideline VDI 4330 Part 10 on floristic mapping

In Central Europe, floristic mapping for scientific purposes looks back on a long history. First prints of local flora books appeared at the end of the 16th century and reached a modern scientific standard already in the 19th century (e.g. Ascherson 1864). For Germany, a milestone at the end of the 20th century was the publication of the results of two floristic mapping projects covering West and East Germany in two separate volumes (Haeupler and Schönfelder 1988, Benkert et al. 1996). Rather comprehensive guidelines concerning the organisation and methods of floristic mapping in Germany

were laid down by the Central Office for Floristic Mapping in Germany in the early 1990s (Bergmeier 1992). The results of floristic mapping are often used in landscape planning and nature conservation, e.g. to set up targeted conservation programmes for particular species or to compile Red Lists of rare and endangered plants (cf. Schulte and Voggenreiter 2000, Sukopp 2001, Haeupler 2005, Sukopp and Sukopp 2006, Sukopp et al. 2006). For example, the data collected during the recent floristic mapping of the city of Hamburg were published together with the latest Red List of vascular plants of the city (Poppendieck et al. 2010). A comprehensive overview of approximately 250 flora books and plant distribution atlases, that were published between 1945 and 2010 covering either the whole country or certain regions of Germany, was compiled by Horn et al. (2006, 2012). Some outstanding examples of recent regional floristic mapping projects are the surveys of the Regnitz river basin (Gatterer and Nezadal 2003), of North Rhine-Westphalia (Haeupler et al. 2003), of the city of Hamburg (Poppendieck et al. 2010) and of the city of Berlin (Seitz et al. 2012). In these projects, mapping was carried out with a systematic approach on the basis of a regular grid and in a highly professional manner.

In close cooperation with the VDI, a group of seven voluntary experts worked on the Guideline VDI 4330 Part 10 between 2008 and 2011. The work aimed at providing standard methods that support a scientifically sound and efficient post-market environmental monitoring of GM plants and their use. The work was embedded in the conceptual framework specified in the Guideline VDI 4330 Part 1 on basic principles and strategies for the environmental monitoring of GM plants (Züghart et al. 2013). The participating experts brought their personal competent view on floristic mapping to the formulation of the new guideline. Furthermore, the committee could build on the previously released VDI Guideline on the assessment of the diversity of ferns and flowering plants by means of vegetation records (Beismann et al. 2008). First, the expert group developed an internal preliminary draft that was then published (Bleeker et al. 2010) and subjected to a public approval procedure (see also Züghart et al. 2013). All received objections were discussed and dealt with by the experts. Finally, the revised Guideline VDI 4330 Part 10 was published (Bleeker et al. 2011).

Contents of the Guideline VDI 4330 Part 10 on floristic mapping

The Guideline VDI 4330 Part 10 is entitled “Floristic mapping of genetically modified plants (GM plants), their crossing partners and their hybrid offspring” (Bleeker et al. 2011). The described floristic mapping methods are based on well-established and widely applied procedures, but needed to be adapted to the specific requirements of post-market environmental monitoring of GM plants and their commercial use. Plants to be recorded are spontaneously occurring (non-cultivated) GM crops, their wild potential crossing partners and hybrid offspring resulting from crosses between GM crops and these partners. Planted or intentionally sown GM crops are not mapped.

The floristic mapping according to Guideline VDI 4330 Part 10 covers only vascular plants. All plants recorded in the area under survey are identified to the rank of species and in the case of aggregates, to the rank of microspecies. Infraspecific taxa (subspecies, varieties, etc.) can be differentiated if this information is required to analyse and interpret the mapping data. Identification is made using standard floras or alternatively in the case of critical taxa with the aid of specialist literature. The nomenclature is based on up-to-date reference publications.

Guideline VDI 4330 Part 10 describes standardised methods for all steps of the floristic mapping procedure. The ten most important issues are (Bleeker et al. 2011):

(1) Equipment and material needed for the field work is listed.
(2) Necessary steps to prepare the field work are explained.
(3) The selection of individual mapping dates and the scheduling of the entire mapping programme in the course of several years are explicated. The plants to be mapped should be recorded at a time when they can be most easily identified. To determine the original state (reference state) before GM plants were introduced into the environment, mapping must begin the year before the first use of GM plants. Mapping is then carried out annually for the entire period of GM plant use. If the use of GM plants is interrupted or terminated, mapping must continue annually for a period of at least eleven years after the last year of GM plant use. Mapping can be terminated earlier, if no spontaneous occurrences of the plants to be mapped are found in four consecutive years following the last year of GM plant use.
(4) Rules are given how to delimit the study area. In the case of crop cultivation, the study area includes fields where GM crops were previously grown and areas of land and paths surrounding current or former fields of GM plants. In the case of transportation, processing or use of GM plants as food or animal feed, areas surrounding the GM plant processing, storage, handling and usage facilities including access routes to and from these facilities must be surveyed. The general spatial design of the mapping consists of at least ten concentric zones at intervals of 0 m to 100 m (zone 1), 100 m to 200 m (zone 2), 300 m to 400 m (zone 3), etc. around current or former fields of GM plants or around processing, storage, handling and usage facilities of GM plants (see Figure 1). At the time of the survey, the zones are systematically examined for the presence of the plants to be mapped, starting at the innermost zone. Main access routes (roads, railway lines and waterways) and drivable paths including their verges are examined as well within the 2000 m wide study area around places where GM plants are cultivated, processed, stored or handled (see Figure 1).
(5) Field recording starts with entering general data, such as identification number, date and name of field observer, in the data sheet.
(6) The location and exact outline of the area covered by the current or former GM plant field or accordingly by the processing, storage, handling and usage facility of GM plants is verified in the field and the boundaries marked on the prepared map are corrected if necessary.

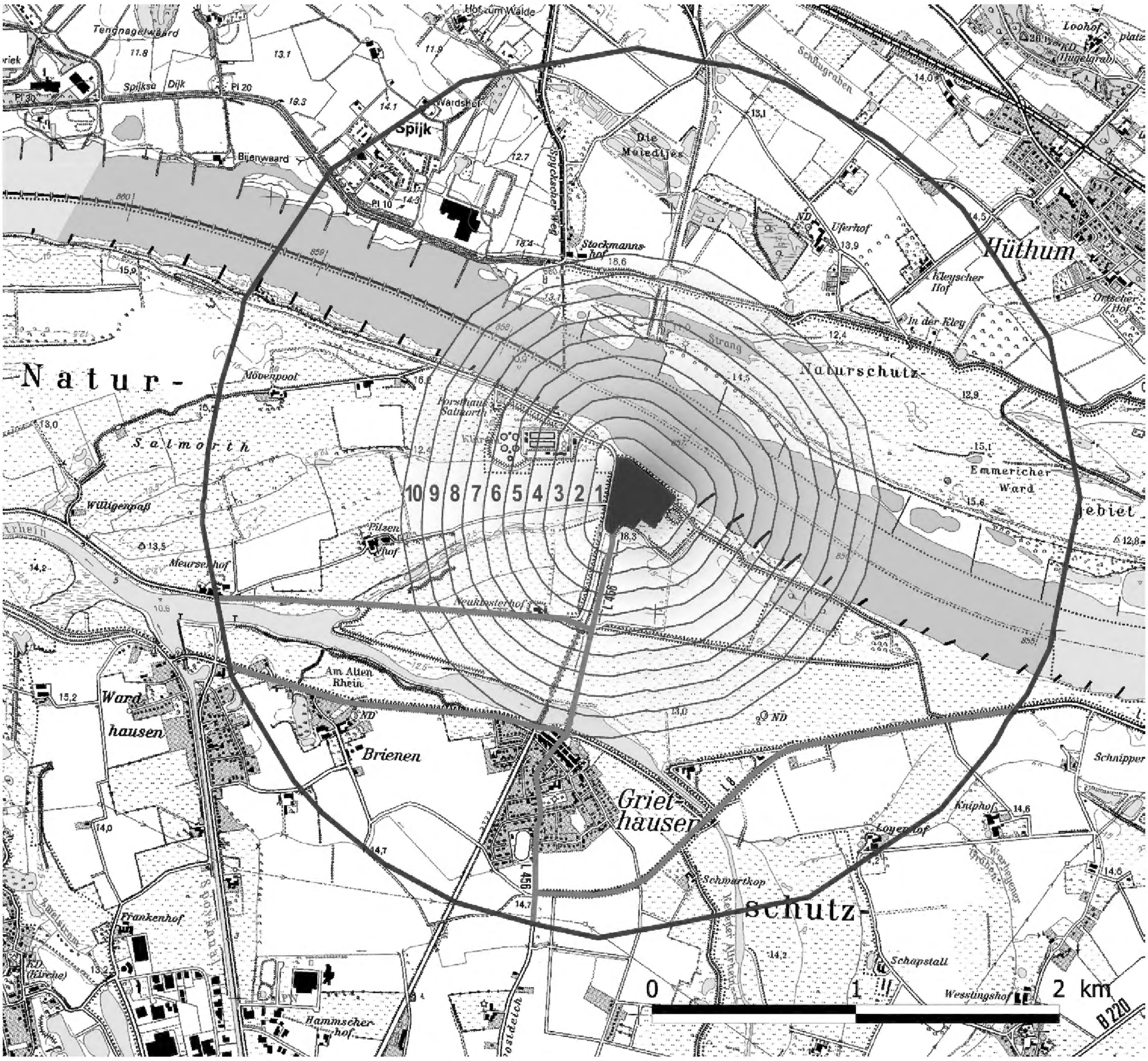

Figure 1. Example for floristic mapping in the surrounding area of a processing facility (Guideline VDI 4330 Part 10, Bleeker et al. 2011). The following elements are marked on a topographic map: **1** the site of an oil mill (blue area) **2** ten concentric zones at intervals of 0 m to 100 m (zone 1), 100 m to 200 m (zone 2), 300 m to 400 m (zone 3), etc. around the site (thin green lines) **3** boundary of the mapped area at a distance of 2000 m around the site (thick green line) **4** main access routes to and from the site within a range of 2000 m around the site (thick red lines) **5** waterway for shipments to and from the site within a range of 2000 m around the site, and up to a minimum distance of 3000 m from the site in the direction of flow, including regularly flooded backwaters and meanders (pink area).

(7) For the floristic mapping, the study area is systematically examined for the presence of the plants to be mapped. Any specimen found is identified as exactly as possible by examining its morphological characters. Usually, it is impossible to distinguish between GM and non-GM plants in the field on the basis of their apparent characters. A reliable distinction can only be made by molecular biological analysis of plant samples in the laboratory. For this reason, plant samples are collected during mapping in accordance with Guideline VDI 4330 Part 5 "Guidelines for the collection and preparation of plant samples for molecular biological analysis" (Belter et al. 2010).

(8) The localities of the plants to be mapped are marked on the prepared maps in the field using point, line and area symbols. The localities are numbered on the map, and the numbers are also entered on the field data sheet. A locality is defined as the smallest distinguishable area that provides a uniform habitat for individuals of the mapped taxon without large gaps (generally < 20 m for a scale of 1: 5000). The plants of one locality must be in a comparable developmental stage. If the individuals of a plant taxon at one site largely differ in their developmental stage, it is required to distinguish the different stages and to quantify the respective numbers of individuals at each developmental stage (see next paragraph). A locality may contain several sampling points.

(9) For each occurrence of plants of one taxon at one locality, the total number of individuals estimated by means of a logarithmic scale (I: 1 individual, II: 2 to 10 individuals, III: 11 to 100 individuals, IV: 101 to 1000 individuals, etc.) is entered on the field data sheet.

(10) The biotope type and – if possible – the vegetation type of the individual localities are entered on the field data sheet. This information is based on a named reference work. Further information about the locality may also be included, e. g. the use of herbicides or the presence of patches of bare soil.

The field data are transferred from the field map to a final map in appropriate scale. GM plants and their hybrid offspring must first be verified by molecular biological analysis. When creating a digital map, all information about the position of occurrences can be processed with GIS software. Tables are prepared containing the names of the recorded taxa arranged by locality number, the corresponding number of individuals, the area size or the length of the locality and the biotope or vegetation type. The mappings can be analysed in terms of the spatial distribution of the localities and the temporal development during the study years.

The documentation includes a description of the methods and all data acquired in the field and from other investigations. Floristic mapping data gathered in accordance with this guideline should be permanently archived in an appropriate database. In Guideline VDI 4330 Part 1, Section 6, the general requirements for documentation and data management are specified.

General discussion and conclusions

Monitoring the environmental effects of GM organisms comprises measurements and observations of elements of communities, habitats and landscapes in regular spatiotemporal sequences that are designed to achieve accurate results on the state and changes of those elements. A suitable monitoring employs scientific methods and is directed towards nature conservation and environmental protection objectives (Sukopp and Weddeling 2007). In the case of viable GM plants entering the environment during cultivation, transport, handling or processing, one important issue of the monitoring are effects

on the local and regional flora. Thus, floristic mapping provides an overview of the frequency and spatial pattern of spread and persistence of GM plants as well as of their potential crossing partners and hybrid offspring. Areas to be mapped are those where the before mentioned plants can spontaneously or subspontaneously occur (Bleeker et al. 2011).

If GM plants are cultivated on arable land, GM volunteers appearing on the fields can have the same effects on the local and regional flora as the formerly cultivated plants (Munier et al. 2012). This particularly applies to cultivated GM oilseed rape that can build up a persistent seed bank. Several studies in Canada and the USA have observed volunteers of GM herbicide resistant oilseed rape that can contribute to subsequent gene flow to other sexually compatible plants in the surrounding area (Knispel et al. 2008, Beckie and Warwick 2010, Schafer et al. 2011, Munier et al. 2012).

The magnitude of potential spread, persistence and outcrossing events strongly depends, among other important factors, on the area cultivated with GM plants and on the amount of GM plants in imported commodities to be processed for further use, e.g. as human food or animal feed. Therefore, it is reasonable to focus the floristic mapping primarily on localities and regions where cultivation, transport, handling or processing takes place (Züghart et al. 2005, 2008, BfN et al. 2011). On the other hand, there is evidence for human-mediated unintentional long distance transport of viable seeds (von der Lippe and Kowarik 2007a, 2007b, Wichmann et al. 2009) that can result in spontaneous growth of GM plants in regions more or less remote from the original localities of cultivation or processing.

Directive 2001/18/EC (EC 2001) and Regulation (EC) No 1829/2003 (EC 2003) stipulate monitoring activities in order to identify adverse effects on human health or the environment caused by GM organisms during commercial use. Therefore, the results of the analysis of monitoring data have to be accurately assessed in order to determine whether observed environmental changes may be classified as adverse effects. For this purpose, a sound concept of environmental damages based on predefined criteria has to be applied (Heink et al. 2007, 2012, Kowarik et al. 2008, Bartz et al. 2009). Adverse environmental effects can only be determined if they are related to certain relevant protection goals (e.g. protection of a natural resource such as a population of an endangered species) (Heink et al. 2007, Kowarik et al. 2008). Descriptions of environmental changes have to be clearly separated from the evaluation of such changes. The first issue is part of a scientific approach, the latter issue depends on normative settings beyond science. It is a matter of politics and society to enact a legal framework and detailed regulations defining relevant environmental and nature protection goals, as it was done e.g. by the Directive 2004/35/EC on environmental liability with regard to the prevention and remedying of environmental damage (EC 2004). Furthermore, criteria have to be agreed on, the application of which leads to a decision whether a certain protection goal is significantly impaired through environmental impacts of GM plant use.

Both approaches, the scientific description of change and the normative assessment of monitoring results, are often erroneously mixed up creating false conclusions. The review of Devos et al. (2012) on feral GM herbicide tolerant oilseed rape from

seed import spills raises the question if concerns about environmental damages are scientifically justified. The question and the attempt of an answer fail to separate scientific analysis from evaluation. On the contrary, Wilkinson and Ford (2007) present a much more sound concept of environmental hazard identification when they introduce so-called endpoint species of conservational significance that could be negatively impacted upon by certain effects of spread, persistence or outcrossing of GM plants.

Persistence and spread of GM crop plants or of their hybrid offspring may also lead to unwanted detrimental effects such as the decline or even replacement of populations of native species. The persistence of herbicide tolerant GM plants or of their hybrid offspring on crop land can also aggravate weed control resulting in much higher quantities of herbicide applications or the need to use mixtures of different herbicides (EFSA 2010). On the long run, this may progressively impoverish the wild flora in agricultural regions or may deteriorate ground water quality. A primarily economic damage could be the impurity caused by GM crops if admixed with non-GM crops of conventional or organic farming. This would induce high costs to implement coexistence measures that guarantee the side-by-side operation of GM and non-GM cropping systems (Devos et al. 2009). Furthermore, it may indirectly hamper environmentally sustainable ways of agriculture and increase the use of herbicides posing higher threats to wild plants and associated insects and other animals on crop land.

One important goal of standardisation is safeguarding the quality of procedures and results. Standardisation of monitoring methods ensures a high level of reproducibility and comparability of the results (Schröder et al. 1991, Berhorn et al. 2005, Beismann et al. 2007, Graef et al. 2007, BfN et al. 2011, Züghart et al. 2013). In case of floristic mapping, this involves testing the plausibility of the mapping data both during collection and on completion of the report. Quality assurance also includes a comparison of the results of the study with those of similar studies. The competence of the field observer and the correct use of the necessary resources and equipment are a fundamental requirement for assuring work quality. The field observer must be able to demonstrate a detailed and up-to-date knowledge of the flora of the ferns and flowering plants of the investigated country or region. The locality of any mapped plant must be precisely recorded. An independent repetition of the mapping can be performed to verify the results (Bleeker et al. 2011).

Yet, we are far away from understanding the environmental impacts of GM plants. Many case studies and even reviews are predominantly based on results deduced from rather fragmentary data or just models about real processes in the landscape. Consequently, more or less imprecise likelihoods substitute clear facts. Thus, data-based evidence from comprehensive and reliable monitoring programmes should be achieved in the future. Standardisation of relevant monitoring techniques can contribute to this goal. The application of the Guideline VDI 4330 Part 10 on floristic mapping provides several advantages in the context of GM plant monitoring:

- The sampling design and the mapping methods are state-of-the-art.
- The mapping strategy focuses on spread, persistence and outcrossing of GM plants originating from one known potential source (e.g. a field or a processing factory).

- The floristic mapping intends to get best results with reasonable effort.
- The spatial and temporal organisation of the floristic mapping is designed to find a balance between the effort in the field and the reliability of data on the presence and – even more important – absence of the surveyed plants.
- The application of standardised methods leads to accurate, comprehensive and reproducible mapping results.
- The guideline provides detailed instructions on data management and quality assurance.
- The application of the guideline contributes to a cost-efficient monitoring of environmental effects of GM plants.
- The guideline on floristic mapping (VDI 4330 Part 10, Bleeker et al. 2011) and the guideline on collection and preparation of plant samples for molecular biological analysis (VDI 4330 Part 5, Belter et al. 2010) are coordinated and should be applied together in order to determine the genetic identity of mapped plants.

In the future, new developments of GM plants or of the way of using GM plants (e.g. new species or varieties, new traits, use in new environments) can give reasons for amending or adjusting the Guideline VDI 4330 Part 10. According to the VDI rules of guideline development, updates can be done at the latest five years after publication when the guideline will be subjected to a revision.

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